Primary Reviewer: Cassandra Kirk, Ph.D., Biologist, Emerging Technologies Branch **Date:** Date: 2020.04.16 10:44:36 -04'00'

Secondary Reviewer: Chris A. Wozniak, Ph.D., Biotechnology Special Assistant, OPP/BPPD **Date:**

DATA EVALUATION RECORD

TEST MATERIAL (PURITY): *Aedes aegypti* OX5034

SYNONYMS: OX5034

CITATION: Proposed Arbovirus qRT-PCR Testing Protocol Outline, Volume 3, EUP Submission; MRID 51094403; March 23, 2020

SPONSOR: Oxitec Ltd, 71, Milton Park, Abingdon, Oxfordshire, OX14 4RX United Kingdom

AUTHOR: Oxitec Ltd.

TEST SITE: Milton Park, Abingdon, Oxfordshire, UK.

COMPLIANCE: Good Laboratory Practice Standards, 40 CFR Part 160, are not applicable to this report. However, the study was conducted according to accepted scientific methods and the raw data and study records have been retained.

This DER does not contain FIFRA CBI.

PURPOSE:

In a 75-day deficiency letter (19 March 2020), EPA requested new arbovirus testing parameters for screening the OX5034 colony for arbovirus infection based on review of MRID 50889425. The letter requested the following:

"Oxitec must submit arbovirus testing data utilizing an RT-PCR method with demonstrated diagnostic validation, quantitative measures of analytical specificity and sensitivity, in addition to appropriate controls and sample size that effectively demonstrate that laboratory colonies are arbovirus (*i.e.* Dengue Virus, Chikungunya Virus, West Nile virus, Saint Louis Encephalitis, Eastern Equine Encephalitis, Western Equine Encephalitis, Venezuelan Equine Encephalitis, Mayaro and Sindbis Viruses) free. A rationale must be provided for detection threshold as well as sample sizes tested. EPA requires testing methods describing the parameters above be submitted now and will require submission of confirmatory data that EPA must review and determine to be acceptable prior to any field release of mosquitoes."

The purpose of this data evaluation record is to review the submission provided by Oxitec in response to the Agency's request.

EXECUTIVE SUMMARY:

The developer of the male-selective *Aedes aegypti* OX5034 mosquito, Oxitec Ltd., used commercially available test kits (VecTOR Test Systems Inc.) to demonstrate the absence of arbovirus infections in the OX5034 production colony. OX5034 mosquitoes were tested for the detection of arboviruses including Dengue, Chikungunya, West Nile Virus (WNV), Saint Louis encephalitis (SLE), eastern equine encephalitis (EEE), Western Equine Encephalitis (WEE), Venezuelan Equine Encephalitis (VEE), Mayaro (MAY) and Sindbis (SIN) viruses. The Agency disagreed with the study author's conclusion that the results of the Vector Test® Systems Inc. arbovirus detection kits used for detection of Chikungunya, Dengue, WNV, SLE, EEE, WEE, VEE, MAY and SIN viruses confirms that the OX5034 production colony is free of arbovirus infection, due to uncertainties regarding the sensitivity and specificity of the assays utilized for arbovirus testing and due to the lack of testing for Zika virus. As a result of this review, EPA requested new arbovirus testing parameters for screening the OX5034 colony for arbovirus infection based on review of MRID 50889425 in a 75-day deficiency letter (19 March 2020).

This submission is considered **SUPPLEMENTAL** for use in the risk assessment because the rationale regarding choosing which arboviruses to test for based upon the presence of the vector and viruses at the release sites is inappropriate for addressing risk of arboviral infection of the source colony located in the UK for the EUP.

CLASSIFICATION: SUPPLEMENTAL

RESULTS:

In response to the Agency's request, Oxitec submitted a rationale detailing which arboviruses they have determined are relevant for screening the OX5034 colony which, included a subset of the arboviruses previously tested for using the VectorTEST® kits **(Table 1.)**. The overall rationale stated "Oxitec proposes conducting qRT-PCR-based arbovirus testing only for viruses that are known to be transmitted by *Aedes aegypti* in the geographic range relevant for releases, *i.e.* FL and TX. These viruses include Dengue and Chikungunya Viruses, but not the Equine Encephalitis Viruses (SLE, EEE, WEE, VEE), West Nile Virus, Mayaro Virus or Sindbis Virus." Oxitec also presented a list of commercial kits available for detection of Dengue, Zika and Chikungunya viruses in serum and/or mosquito samples. These include, but are not limited to, those presented in **Table 2.** Oxitec proposes the use of commercially available multiplex qRT-PCR testing kits for detection of Dengue (Serotypes 1-4), Chikungunya and Zika viruses, based on the relevance of *Aedes aegypti* for the transmission of these viruses. Oxitec also proposes a sample size of 900 mosquitoes, analyzed as pooled samples of 10 mosquitoes, equating to a detection threshold of 0.3% infection in the Oxitec OX5034 mosquito colony. They stated that assay specificity and sensitivity would not be re-determined as the assays cited are commercially available validated assays. Viral RNA sequences would be used as controls.

Table 1. Arbovirus detection kits for mosquito samples used by Oxitec for OX5034 mosquito analysis (MRID 50889425) and *Aedes aegypti* vectoring status

VectorTest Kit Catalog Number	Arbovirus	Transmitted by *Aedes aegypti*	References
DEN-K050	Dengue Virus (all four serotypes)	Yes	(Weaver & Reisen, 2010)
WSE-K050	West Nile Virus	Unlikely (some lab-based evidence); main vectors are *Culex* spp.	(Weaver & Reisen, 2010; Joubert & O'Neill, 2017; Chapman et al., 2018)
	Saint Louis Encephalitis Virus	No. Transmitted by *Culex spp.*	https://www.cdc.gov/sle/technical/transmission.html
	Eastern Equine Encephalitis Virus	No. Transmitted by *Aedes taeniorhynchus*.	(Chapman et al., 2018)
ALP-K050	Chikungunya Virus	Yes	(Pezzi et al., 2020)
	Eastern Equine Encephalitis Virus	No. Transmitted by *Aedes taeniorhynchus*.	(Chapman et al., 2018)
	Western Equine Encephalitis Virus	No. Transmitted by *Ochlerotatus melanimon*, *Culex tarsalis* and other *Aedes* spp., not *Aedes aegypti*.	(Aréchiga-Ceballos & Aguilar-Setién, 2015; Chapman et al., 2018)
	Venezuelan Equine Encephalitis Virus	No. Transmitted by *Psorophora* and *Ochlerotatus* spp.	(Chapman et al., 2018)
	Mayaro Virus	Unlikely. Major host is *Haemagogus* sp. and no wild transmission known (laboratory studies only). Only present in central/South America.	(Pezzi et al., 2020)
	Sindbis Virus	Rarely (laboratory studies), main vectors are *Culex* spp., not present in North/ South America.	(Muturi et al., 2011; Adouchief et al., 2016; Chapman et al., 2018)

Table 2. Commercial and published kits/assays for arbovirus detection.

Supplier	Assay	Controls Supplied	Analyte Validated (Serum/Mosquito)	References
ThermoFisher	TaqMan Zika Virus Triplex Vector Screening Kit (ZIKV/DENV/CHIKV)	No	Mosquito	Lura and Brown, 2019
BioRad	Zika, Dengue, and Chikungunya (ZDC) Real-Time PCR Assays	Yes (Dengue, Zika and Chikungunya RNA sequences)	Mosquitoes and serum	https://www.biorad.com/webroot/web/pdf/lsr/literature/10000071135_digital_lock.pdf
Altona Diagnostics	RealStar® Zika Virus RT-PCR Kit 1.0 (WHO validated) RealStar® Dengue Virus RT-PCR Kit 2.0 RealStar® Chikungunya Virus RT-PCR Kit 2.0	Yes (Dengue, Zika and Chikungunya RNA sequences)	Serum	L'Huillier et al., 2017; Mat Jusoh and Shueb, 2017; Heitmann et al., 2018
CDC	Trioplex Real-time RT-PCR Assay	Inactivated virus samples for each virus (CDC; catalogue #KT0167)		https://www.cdc.gov/zika/pdfs/trioplex-real-time-rt-pcr-assay-instructions-for-use.pdf

Study Author's Conclusion:
The study author concludes that taken together, the use of qRT-PCR assays for the detection of arboviruses vectored only by *Aedes aegypti* which, are present in the EUP release areas (*i.e.* FL and TX) would be sufficient to confirm that the OX5034 colony is free of arbovirus infection.

Reviewer's Comments:
In response to the Agency's request, Oxitec submitted a rationale detailing which arboviruses they have determined are relevant for screening the OX5034 colony which included a subset of the arboviruses previously tested for using the VectorTEST® kits. The tests carried out and reported in MRID 50889425 used three kits available from VectorTEST® Inc. Oxitec purported that not all of the viruses detectable by each kit are vectored by *Aedes aegypti.* The ability of the kits to detect multiple viruses is a result of the similarity of the viral antigen protein detected (*e.g.* Chikungunya, transmitted by *Aedes aegypti*, has similar genetic sequence to other alphaviruses like EEE Virus, which is not transmitted by *Aedes aegypti*).

The test kits previously purchased by Oxitec from VectorTEST® Inc were designed to detect a suite of arboviruses of medical importance (*i.e.* the most prevalent arboviruses in the US that are of human health concern). These assays can simultaneously detect arboviruses transmitted by *Aedes aegypti* in addition to other vectors. The Agency agrees with Oxitec that arboviruses vectored by mosquito species other than *Aedes aegypti* are not relevant for screening because research and data indicate that *Aedes aegypti* are only competent at transmitting a limited number of arboviruses.

***Aedes aegypti* vector competency**
Arthropod-borne viruses (arboviruses) are important causes of human disease nearly worldwide (Weaver and Reisen, 2010). The four viruses that have had the greatest impact on human health for which *Aedes aegypti* is the primary vector include the Yellow Fever Virus, Dengue Viruses, Chikungunya Virus, and Zika Virus (Souza-Neto et al., 2019). A few studies have demonstrated that *Aedes aegypti* can be infected with other arboviruses in addition to these four viruses, however, transmission of the virus to other hosts (*e.g.* humans, wildlife) once the mosquito is infected was found to be unlikely. For example, laboratory studies to examine the impact of *Wolbachia* on the replication of West Nile Virus in *Aedes aegypti* demonstrated that *Aedes aegypti* can be experimentally infected by West Nile Virus, however, no infectious virus was detected in saliva from these mosquitoes (Joubert and O'Neill, 2017). Major vectors for West Nile Virus include many *Culex spp.*; some of the most important include *Culex pipiens, tarsalis, modestus,* and *quinquefasciatus*, not *Aedes aegypti* (Chapman et al., 2018).

Serra et al. (2016), found non-engorged *Aedes aegypti* naturally infected with Mayaro Virus in the city of Cuiaba, a central region of Brazil, however either no or poor infection and dissemination rates were demonstrated when orally challenged with artificial blood meals containing Mayaro Virus at titers similar to those usually found in infected humans (Brustolin et al., 2018; Long et al., 2011), which may limit chances to initiate an urban transmission cycle. However, *Aedes aegypti* were able to experimentally transmit Mayaro Virus when taking a blood meal with high viral load; therefore, the authors concluded that the hypothesis that *Aedes aegypti* could contribute to urban transmission should not be completely neglected (Long et al., 2011). In addition, *Aedes aegypti* from Florida State (Wiggins et al., 2018) and from Iquitos (Peru) (Long et al., 2011) were competent to transmit Mayaro Virus in laboratory conditions. Major vectors of Mayaro Virus include *Haemagogus sp.* No wild transmission of Mayaro Virus by *Aedes aegypti* has been found to date (Pezzi et al., 2020).

A laboratory study conducted by Muturi et al. (2011) used *Aedes aegypti* as a model species to examine the effect of larval stress on vector competence for Sindbis Virus found that stress during larval development may cause alterations in adult mosquito phenotype and immunity that can increase their susceptibility to pathogens. This study demonstrates that *Aedes aegypti* can be experimentally infected with Sindbis Virus in the laboratory, however it is not a major vector of the virus. Major vectors for Sindbis Virus include *Culex spp.* and *Culiseta spp.* (Chapman et al., 2018).

***Aedes aegypti* geographic range and surveillance in the UK**
The reviewer disagrees that testing for viruses that are known to be transmitted by *Aedes aegypti* in the geographic range relevant for releases, *i.e.* FL and TX, is appropriate because the risk for arbovirus infection of the source colony would occur in the UK where the colony is maintained, not the release sites. For the EUP the colony will be maintained at Oxitec's insectary in Milton Park, UK and OX5034 eggs will be shipped to rearing facilities in the US for deployment to release sites. It should be noted that no colony will be maintained in the US for the EUP (For more information on the description of starting materials, production and formulation process see MRID 50889424). The risk for colony infection is based on the presence of *Aedes aegypti* and the occurrence of the arboviruses the mosquito is capable of vectoring in the UK.

The mosquito *Aedes aegypti* is found in tropical and sub-tropical regions where it is the major vector of Dengue Virus, Yellow Fever Virus, Chikungunya Virus and more recently, Zika Virus. Given the importance of *Aedes aegypti* as a vector of arboviruses and its propensity to be transported to new regions, the European Centre for Disease Prevention and Control (ECDC) has placed *Aedes aegypti* on a list of potentially invasive mosquito species. Public Health England (PHE) runs the nationwide mosquito surveillance project which, conducts surveys for invasive mosquitoes in the UK. The UK currently has no known established populations of any invasive *Aedes* spp. mosquitoes (Medlock et al., 2019). *Aedes aegypti* was previously reported in the United Kingdom (UK) in 1865 and 1919 but did not establish on either occasion. More recently, it has reappeared in European countries and has been recorded in the Netherlands (not established) and Madeira (Portugal), as well as southern Russia, Georgia and Turkey (Dallimore et al., 2017). In 2014, a single male *Aedes aegypti* was found in Merseyside, England, however follow-up surveys determined that there was no established population (Dallimore et al., 2017).

Absence of arboviruses vectored by *Aedes aegypti* in the UK

None of the arboviruses (Dengue Virus, Chikungunya Virus, Yellow Fever Virus and Zika Virus) for which *Aedes aegypti* is a major vector occur naturally in the UK. The most recent data in the European Centre for Disease Prevention and Control's (ECDPC) Surveillance Atlas for Infectious Disease show no locally acquired cases of these viruses in the UK (ECDPC, 2017). While arbovirus infections have been reported in the UK, these were acquired while on travel outside of the country. In recent years a small number of travel-related Chikungunya cases have been reported in England, Wales and Northern Ireland (EWNI) annually. Most have been acquired in the Indian sub-continent and South East Asia. In 2014, 295 cases of Chikungunya were reported in EWNI of which 88% had acquired their infection in the Caribbean and South America (Public Health England, 2014). Cases of Dengue in UK travelers are increasing, with most reported in travelers who visited Asia, the Americas and the Caribbean (Public Health England, 2014). In 2017, there were 465 cases of imported Dengue (ECDPC, 2017).

No vector-borne locally acquired Zika Virus disease cases were reported by EU/EEA countries in Europe as of week 12, 2019 (ECDPC, 2019). According to a risk assessment for Zika Virus released in April 2019 by the ECDPC, between 2015 and week 12 of 2019, 22 EU/EEA Member States reported 2 398 travel-associated Zika Virus infections through the European Surveillance System. France reported 48% of the cases, Spain 15% and the UK, 9%. In 2017, the ECDPC reported one travel-related case of Yellow Fever in the Netherlands (ECDPC, 2017).

Reviewer's Conclusion and Recommendation:

For the EUP the colony will be maintained at Oxitec's insectary in Milton Park, UK and OX5034 male eggs will be shipped to rearing facilities in the US for deployment to release sites The Agency concludes that because neither *Aedes aegypti* nor the arboviruses for which it is a vector are present in the UK, the risk of infection of arbovirus infection of the source colony is low. Therefore, arbovirus testing for the EUP is not required. It should be noted that because testing is not required for the EUP, the test kits cited in **Table 1**. were not reviewed in detail and therefore a determination regarding the adequacy of the proposed testing protocol outline has not been made at this time.

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